

TRK1 and *TRK2* Encode Structurally Related K⁺ Transporters in *Saccharomyces cerevisiae*

CHRISTOPHER H. KO AND RICHARD F. GABER*

Department of Biochemistry, Molecular Biology and Cell Biology,
Northwestern University, Evanston, Illinois 60208

Received 20 March 1991/Accepted 22 May 1991

We describe the cloning and molecular analysis of *TRK2*, the gene likely to encode the low-affinity K⁺ transporter in *Saccharomyces cerevisiae*. *TRK2* encodes a protein of 889 amino acids containing 12 putative membrane-spanning domains (M1 through M12), with a large hydrophilic region between M3 and M4. These structural features closely resemble those contained in *TRK1*, the high-affinity K⁺ transporter. *TRK2* shares 55% amino acid sequence identity with *TRK1*. The putative membrane-spanning domains of *TRK1* and *TRK2* share the highest sequence conservation, while the large hydrophilic regions between M3 and M4 exhibit the greatest divergence. The different affinities of *TRK1 trk2Δ* cells and *trk1Δ TRK2* cells for K⁺ underscore the functional independence of the high- and low-affinity transporters. *TRK2* is nonessential in *TRK1* or *trk1Δ* haploid cells. The viability of cells containing null mutations in both *TRK1* and *TRK2* reveals the existence of an additional, functionally independent potassium transporter(s). Cells deleted for both *TRK1* and *TRK2* are hypersensitive to low pH; they are severely limited in their ability to take up K⁺, particularly when faced with a large inward-facing H⁺ gradient, indicating that the K⁺ transporter(s) that remains in *trk1Δ trk2Δ* cells functions differently than those of the *TRK* class.

To satisfy the cell's requirement for a cytoplasm rich in potassium and to mediate the generation of ion gradients across plasma membranes, multiple routes of K⁺ transport appear to have evolved in many organisms. In higher eukaryotes, the Na⁺/K⁺-ATPase and tissue-specific K⁺ channels accommodate the transport of potassium (20, 29, 34). In prokaryotes, at least four functionally independent K⁺ transport systems have been identified genetically (38). In the yeast *Saccharomyces cerevisiae*, dual affinities for K⁺ uptake (26) have been shown to originate from distinct transporters (10, 21).

The complement of K⁺ transporters present in the cells determines the concentration of extracellular potassium at which cell growth is limited. Wild-type cells can grow in medium containing less than 0.1 mM KCl (3, 26). This phenotype, designated Trk⁺, is conferred by the activity of the high-affinity K⁺ transporter, *TRK1* (10, 25). In contrast, cells containing only the low-affinity transporter (*trk1Δ TRK2*) exhibit a K⁺ low affinity phenotype; they require 3 to 5 mM KCl for growth (10). In *trk1Δ* cells, recessive *trk2* mutations confer a K⁺ low affinity phenotype; i.e., they show an increased potassium requirement (50 to 100 mM) and are unable to grow in acidic medium (pH <4.5) unless supplemented with very high concentrations of potassium (>400 mM) (21). Previous results describing both conditional (21) and dominant (37) mutations at *TRK2* further supported the hypothesis that this locus encodes a structural gene required for low-affinity K⁺ transport. The K⁺ low affinity phenotype of *trk2* cells is completely suppressed by the wild-type *TRK1* gene; growth of *TRK1 trk2* and *TRK1 TRK2* cells in medium containing a minimal concentration of K⁺ (0.2 mM) is indistinguishable (21).

In this report, we describe the cloning of *TRK2*, its molecular analysis, and the effect of *trk2* null alleles. *TRK2* is capable of encoding an 889-amino-acid protein that shares

significant amino acid sequence identity with *TRK1*, and the predicted structural features of the two proteins are remarkably similar. The sequence conservation between *TRK1* and *TRK2* suggests that these genes arose from a duplication event. Although K⁺ uptake is further decreased in *trk1Δ trk2Δ* cells, these mutants are viable, revealing the existence of an additional K⁺ transporter(s). We present data to show that the K⁺ transporter(s) that remains in *trk1Δ trk2Δ* cells is functionally dissimilar to members of the *TRK* family.

MATERIALS AND METHODS

Strains and media. Yeast and *Escherichia coli* strains used in this study are listed in Table 1. YPD, YNB, and sporulation media and standard genetic techniques are described by Sherman et al. (33). Low-salt (LS) and low-pH medium were prepared as previously described (21). The K⁺ concentration of a medium is indicated as millimolar KCl; for example, YPD(100K) is YPD + 100 mM KCl. Yeast transformation was performed by the cation method (19) with lithium acetate.

Construction of a yeast genomic library. Genomic DNA was prepared from a *trk1Δ* strain (R1155) as previously described (9) and partially digested with *Sau3A*. DNA molecules larger than 8 kb were eluted from an agarose gel and ligated to YCp50 that had been linearized with *Bam*HI and treated with calf intestine alkaline phosphatase. A portion of the ligation mixture was used to transform HB101 to ampicillin resistance, and the insert frequency was determined by tetracycline sensitivity testing. The ligation mixture was used to transform a *trk1Δ trk2-3 ura3-52* recipient strain to Ura⁺.

DNA sequencing. pCK52, pCK57, and pCK60 were constructed by subcloning the 1.8-kb *Sal*I-*Xba*I, 2.0-kb *Cl*aI-*Bam*HI, and 0.3-kb *Bam*HI-*Xba*I fragments from pCK33 into pGEM4Z, pGEM7Z, and pGEM4Z, respectively. Sets of nested deletions (17) from pCK52 and pCK57 were generated by using a commercially available kit (Pharmacia),

* Corresponding author.

TABLE 1. Bacterial and yeast strains used

Strain	Genotype	Reference
HB101	<i>hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-15 mtl-14 supE44</i>	19
R1155	<i>MATα his4-15 lys9 ura3-52 trk1Δ</i>	9
R1174	<i>MATα trp1Δ1 ura3-52 trk1Δ</i>	9
R1320	<i>MATα his4-15 lys9 ura3-52 trk1Δ trk2-3</i>	18
CY54	<i>MATα his4-15 lys9 ura3-52 trk1Δ trk2-3::pCK34</i>	This study
A303	<i>MATα his3Δ200 ura3-52 leu2Δ trp1Δ1</i>	This study
M398	<i>MATα his3Δ200 ura3-52 leu2Δ trp1Δ1 trk1Δ</i>	32
Cx142	<i>MATα/MATα his3Δ200/his3Δ200 ura3-52/ura3-52 trk1Δ/trk1Δ</i>	This study
CY218	<i>MATα his3Δ200 ura3-52 leu2Δ trp1Δ1 trk2Δ::pCK64</i>	This study
M469	<i>MATα his3Δ200 ura3-52 leu2Δ trp1Δ1 trk1Δ trk2Δ::pCK64</i>	This study

and both strands of the appropriate clones were sequenced by the dideoxy chain termination method (30) by using Sequenase (United States Biochemical Corp.). Sequence analyses were performed with the DNA Inspector IIe software program (Textco). Protein sequence comparisons of *TRK1* and *TRK2* were generated by using the UWGCG program (5).

DNA manipulations. Preparation of DNA, restriction endonuclease analysis, gel electrophoresis, and Southern blot analysis were as described by Maniatis et al. (23). DNA probes were prepared by the random priming method (7, 8).

Construction of an integrative plasmid. Plasmid pCK34 was constructed by subcloning a 2.2-kb *Bam*HI-*Cla*I fragment of pCK33 into YIp5 and was linearized with *Mlu*I to enhance the frequency of integration.

Construction of *TRK1 trk2 Δ* and *trk1 Δ trk2 Δ* strains. A large deletion of *TRK2* was constructed by the "gamma" deletion method (35). The 0.6-kb *Eco*RV-*Cla*I and 1.5-kb *Bam*HI fragments from pCK33 were subcloned into the integrative shuttle vector pRS303 in which the *Xba*I site had been destroyed, yielding the *TRK2*-deleting plasmid pCK64. *TRK1 trk2 Δ* and *trk1 Δ trk2 Δ* strains were generated by transforming *TRK1 TRK2* and *trk1 Δ TRK2* strains, respectively, with pCK64 linearized by digestion with *Eco*RV and *Xba*I.

K⁺ uptake assays. K⁺ uptake assays were performed essentially as previously described (10, 21). Briefly, cultures of isogenic *TRK1 TRK2*, *TRK1 trk2 Δ* , *trk1 Δ TRK2*, and *trk1 Δ trk2 Δ* strains (A303, CY218, M398, and M469) (Table 1) were grown to saturation in YPD(100K) medium. Cells were harvested, washed twice with double-distilled H₂O by centrifugation, and starved in buffer containing 50 mM Tris-succinate, pH 5.9, for 16 h. After starvation, cells were harvested and suspended in 50 mM Tris-succinate, pH 5.9, to a density of 12,000 Klett units. Glucose was added to a final concentration of 4% at the start of the assay. K⁺ uptake was measured with a K⁺-specific electrode (Orion 931900).

Nucleotide sequence accession number. The nucleic acid sequence of the fragment containing *TRK2* has been deposited in GenBank (accession number M65215).

RESULTS

Cloning of *TRK2*. As described in the introduction, in *trk1 Δ* cells, recessive *trk2* mutations confer both an increased potassium requirement and hypersensitivity to low pH, both hallmarks of the *Kla*⁻ phenotype (Fig. 1). Our strategy to clone the wild-type *TRK2* gene was to screen existing yeast libraries for plasmids that suppressed the *Kla*⁻ phenotype of *trk1 Δ trk2-3* recipient cells. Although, as

anticipated, these screens yielded *TRK1* clones, we failed to obtain the *TRK2* gene despite exhaustive screens of both single-copy (27) and multicopy (4) *S. cerevisiae* libraries. The *TRK1* clones were identifiable by their *Trk*⁺ phenotype (the ability of the cell to grow on a medium containing only 0.2 mM KCl) and their diagnostic restriction site patterns.

We considered that the *TRK2* gene might be underrepresented in the existing yeast libraries and resorted to cloning *TRK2* by a direct yeast-to-yeast transformation method that initially avoids propagation of plasmids in bacteria (9; see Materials and Methods). A ligation mix of *Bam*HI-digested vector (YCp50) and partially digested *Sau*3A genomic fragments from a *trk1 Δ TRK2* strain (R1155) was used to transform a *trk1 Δ trk2-3* recipient (R1320) to Ura⁺. Among approximately 3,000 yeast transformants screened, 2 exhibited the *Kla*⁺ phenotype, i.e., they grew on YNB media containing 7 mM potassium (Fig. 1). These transformants were also capable of growing on a low-pH medium, YPD(100K), pH 4.0 (see Materials and Methods).

Plasmid DNA (pCK33; Fig. 2) was retrieved from one of these transformants by transformation of *E. coli* to ampicillin resistance. Bacterial colonies that harbored pCK33 grew very slowly, and plasmid DNA yields from these cells were extremely poor (22), indicating that the cloned yeast DNA is toxic to *E. coli*. Reintroduction of pCK33 into a *trk1 Δ trk2-3* yeast recipient (R1320) conferred a *Kla*⁺ phenotype on all transformants.

A plasmid integration experiment was performed to show

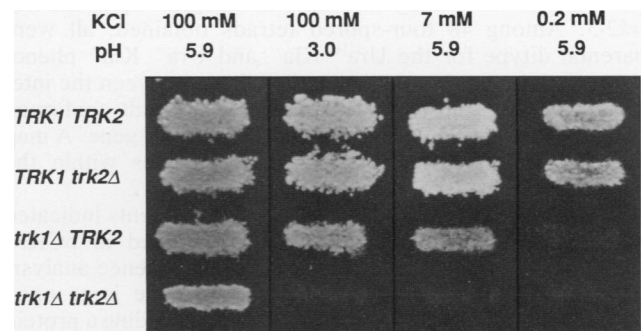


FIG. 1. Growth phenotypes of four isogenic strains containing *trk1 Δ* and *trk2 Δ* mutations. Patches of cells were replica plated onto the indicated media and incubated at 30°C for 2 days before being photographed. YNB media supplemented with KCl and HCl were prepared as described in Materials and Methods. *Trk*⁺ and *Kla*⁺ phenotypes are illustrated by the growth patterns of *TRK1 TRK2* and *trk1 Δ TRK2* cells, respectively.

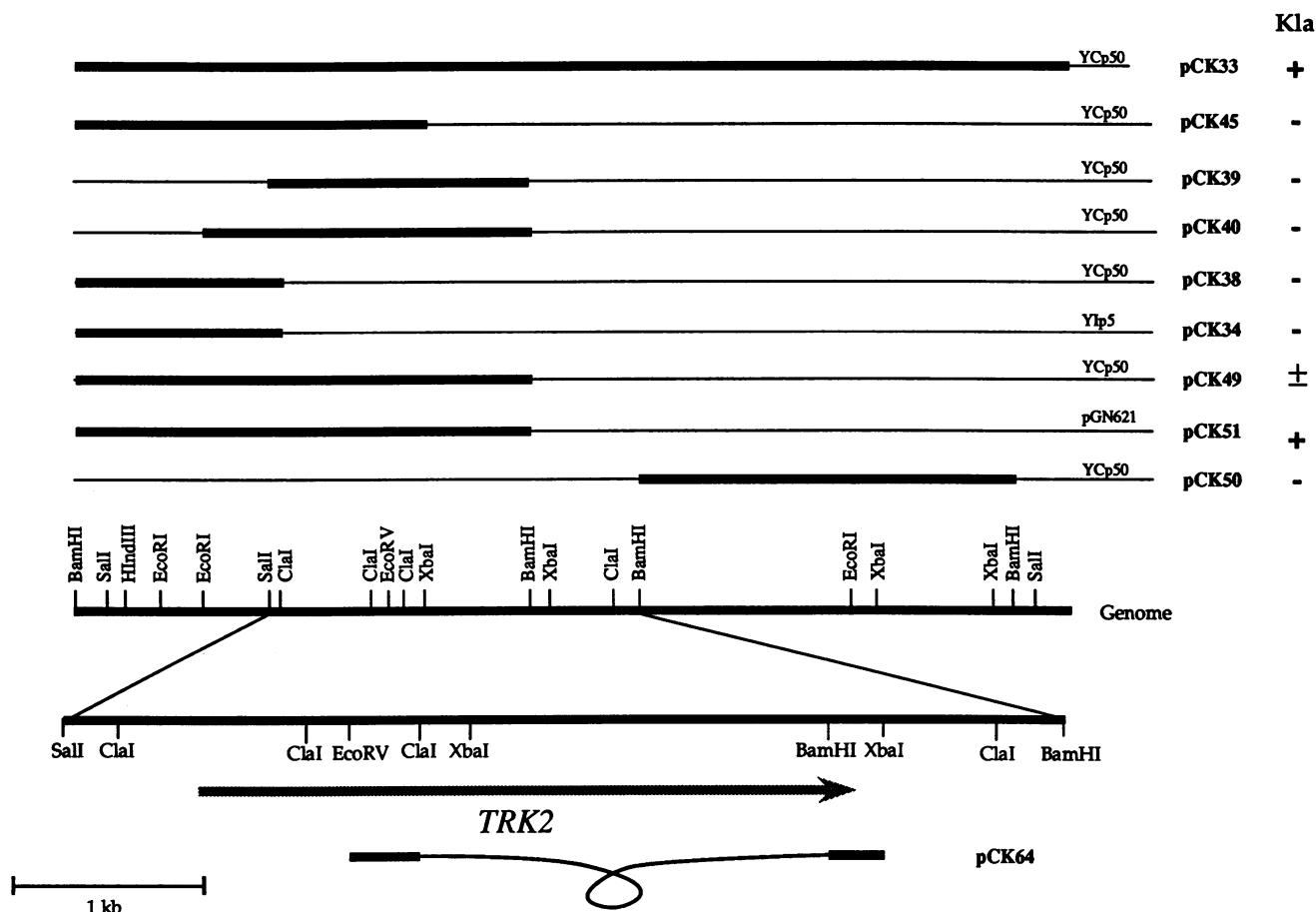


FIG. 2. Restriction maps of plasmids containing the *TRK2* gene and subclones. The thin lines represent vector sequences and the thick lines represent yeast DNA sequences aligned with the restriction map below. The open reading frame of *TRK2* is indicated by a heavy arrow. Subclones were constructed in the vectors indicated and introduced into a *trk1Δ trk2Δ* recipient by selecting for *Ura*⁺. Transformants were tested for growth on YNB(7K) and YPD(100K), pH 4.0. pGN621 is a multicopy vector. pCK64 was used to generate the *trk2Δ* mutation described in the text. The scale refers to the expanded portion of the figure, which is enlarged twofold.

that the cloned DNA fragment contained the *TRK2* gene. pCK34 (Fig. 2; see Materials and Methods) was used to transform a *Ura*⁻ *Kla*⁻ recipient (R1320). One of the *Ura*⁺ *Kla*⁻ transformants was crossed with a *Ura*⁻ *Kla*⁺ strain (R1174) to detect linkage between the integrated plasmid and *trk2-3*. Among 48 four-spored tetrads obtained, all were parental ditype for the *Ura*⁺ *Kla*⁻ and *Ura*⁻ *Kla*⁺ phenotypes, indicating complete genetic linkage between the integrated plasmid and the *trk2-3* locus. This result confirmed that plasmid pCK33 carries the authentic *TRK2* gene. A map of the relevant restriction endonuclease sites within the cloned insert from pCK33 is presented in Fig. 2.

DNA sequence of *TRK2*. Subcloning experiments indicated that the 3.8-kb *SalI*-*XbaI* fragment contained in pCK33 harbors the functional *TRK2* gene. DNA sequence analysis performed on this fragment revealed a single large open reading frame (data not shown) capable of encoding a protein of 889 amino acids with a molecular mass of 101 kDa (Fig. 3). The first 33 N-terminal amino acids of the open reading frame constitute a largely hydrophilic domain and are thus unlikely to function as a signal sequence for vectorial insertion across membranes. Highly charged domains within the protein are found at position 322 to 345 and at position 693 to 734. Asn-X-Ser/Thr sequences at position 216 to 218, 233 to

235, 265 to 267, 606 to 608, 701 to 703, and 801 to 803 indicate sites of potential N-linked glycosylation. Six acidic residues are found within the putative transmembrane domains. The relevance of these residues in *K*⁺ uptake is discussed later.

The 6-kb *Bam*HI fragment containing a slightly truncated *TRK2* gene only weakly suppressed the *Kla*⁻ phenotype of *trk1Δ trk2Δ* cells when expressed from the centromeric plasmid pCK49. This fragment contains a carboxy-terminal 24-amino-acid deletion *TRK2*. The *Kla*⁻ phenotype was completely suppressed, however, when the truncated gene was expressed from a multicopy plasmid (pCK51), indicating that the *TRK2* protein can be overexpressed on such a plasmid and that the carboxy-terminal 24 amino acids of *TRK2* are not essential for its function.

A hydrophilicity plot (18) generated from the predicted *TRK2* sequence identified 12 domains (M1 through M12) of sufficient hydrophobicity and length (6) to be considered potential membrane-spanning domains (Fig. 4). Located between M3 and M4 is a large hydrophilic region 334 amino acids in length. These structural features closely resemble those of *TRK1*, the high-affinity *K*⁺ transporter.

***TRK2* is structurally related to *TRK1*.** A comparison of the inferred amino acid sequences indicates that *TRK1* and *TRK2* are 55% identical (Fig. 3). When conservative substi-

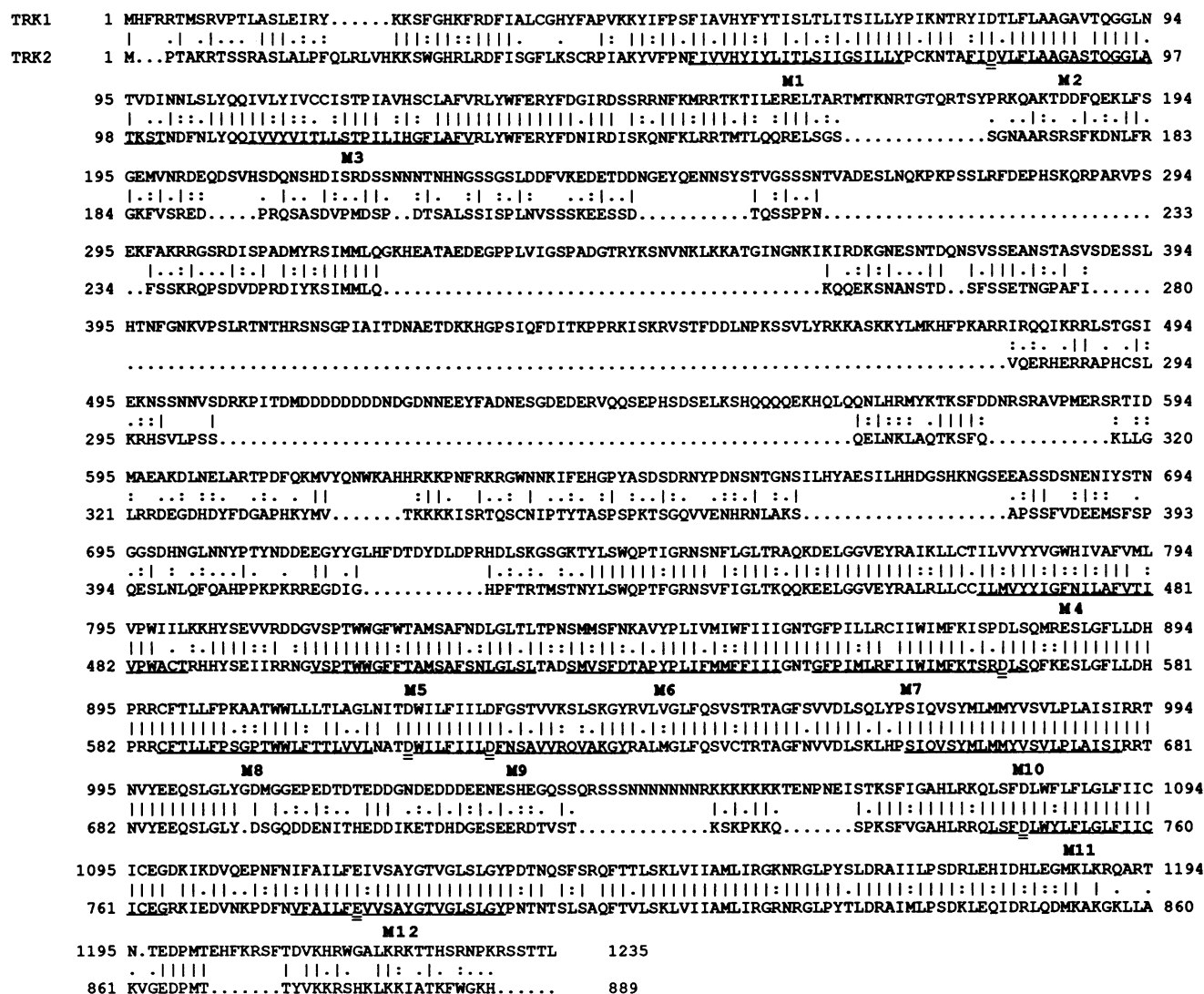


FIG. 3. Alignment of deduced amino acid sequences of TRK1 and TRK2. The alignment was generated by using the GAP program of the University of Wisconsin Genetics Computer Group sequence analysis package (straight lines indicate amino acid identity; two dots indicate conservative substitutions; single dots indicate semiconservative substitutions). The potential transmembrane domains (M1 through M12) are underlined and the conserved acidic amino acids found within or near the putative transmembrane domains are underlined twice in the TRK2 sequence. The TRK1 sequence is from reference 10.

tutions are taken into account, the overall relatedness of these two transporters approaches 71%. The highest degree of sequence conservation is found in the potential transmembrane domains of the two transporters; the identities within these regions range from 55 to 100% (Fig. 3). When the hydrophilicity plots of the two protein sequences are compared, they are almost identical (Fig. 4), indicating that the overall topologies of TRK1 and TRK2 in the plasma membrane may be very similar. Consistent with this hypothesis, 13 of 16 proline residues (likely to confer significant structural constraints) found within or near the putative transmembrane domains of TRK1 are conserved in TRK2 (Fig. 3).

The following structural features found in TRK1 appear to have been lost or diminished in TRK2, suggesting that these genes either evolved from a common ancestral gene or arose as a duplication event: (i) The 650-amino-acid region located

between M3 and M4 in TRK1 is reduced to 334 amino acids in TRK2, and the primary sequences of these regions are almost unrelated; (ii) a highly hydrophilic 27-amino-acid region beginning at Q¹⁰⁴⁰ in TRK1 is reduced to 7 amino acids in TRK2; (iii) the carboxy terminus of TRK2 appears to be reduced by 13 amino acids; (iv) a putative nucleotide-binding domain, GSGKT, contained in the M3-M4 hydrophilic region of TRK1 (10) is either deleted or completely divergent in TRK2; (v) only 2 of 14 sites of potential N-linked glycosylation in TRK1 are conserved in TRK2 (positions 606 and 801); and (vi) among 10 cysteine residues found in TRK1, only 4 (C⁴⁶³, ⁵⁸⁴, ⁷⁶⁰, and ⁷⁶²) are conserved in TRK2.

Construction of *TRK1 trk2Δ* cells. To determine if the high-affinity K⁺ transporter can function in cells deleted for the low-affinity transporter, a *trk2* null mutation was introduced into *TRK1* cells. Plasmid pCK64, containing a 2-kb

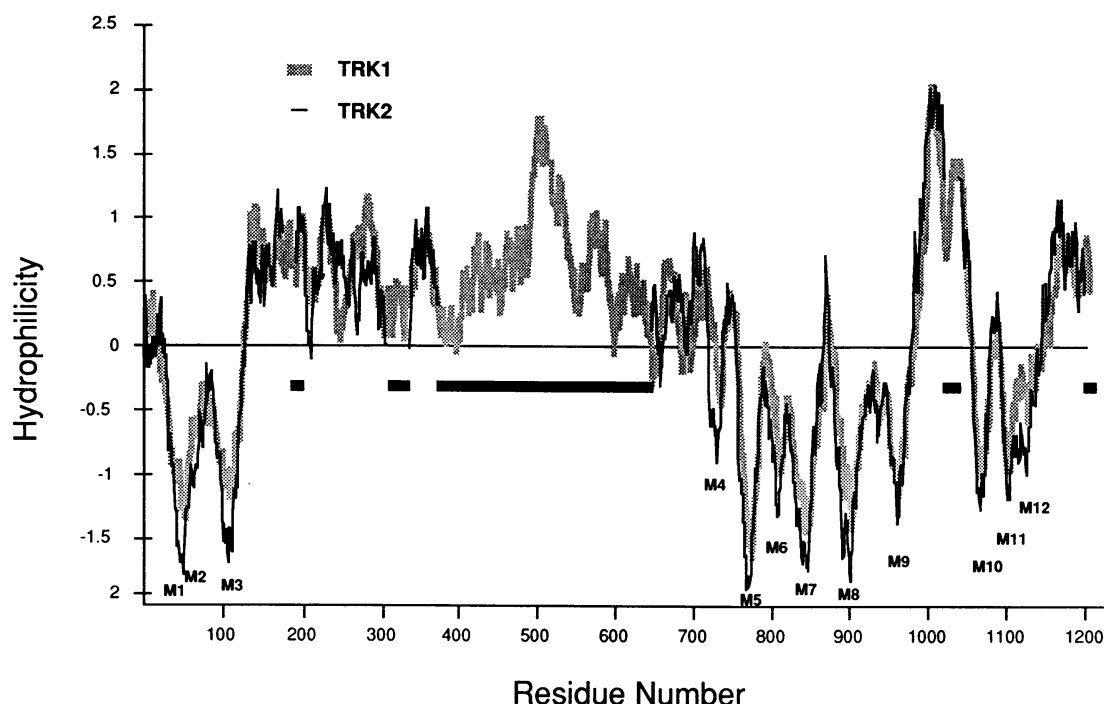


FIG. 4. A comparison of hydrophilicity plots of TRK1 and TRK2. The hydrophilicity plots were generated by the DNA Inspector program by using a 20-amino-acid window size. Horizontal solid bars indicate deletions found in TRK2 (see text). The potential transmembrane domains are indicated by M1 through M12.

deletion within the coding region of TRK2, was used to generate a one-step *trk2Δ* mutation (35) by transforming a *TRK1 TRK2 his3Δ200* recipient (A303) to His⁺ (Fig. 2; Materials and Methods). Southern blot analysis of genomic DNA prepared from one of these transformants (CY218) confirmed that the cells carried the *trk2Δ* mutation (Fig. 5). *TRK1 trk2Δ* cells are phenotypically indistinguishable from *TRK1 TRK2* wild-type cells in their ability to grow on minimal concentrations of potassium (Fig. 1) and their ability to take up K⁺ from the medium (Fig. 6), demonstrating the functional independence of *TRK1* from *TRK2*. The phenotypic masking effect of *TRK1* also explains why *trk2* mutants were not identified in earlier screens (10, 25).

Construction of *trk1Δ trk2Δ* cells. To determine if the low-affinity K⁺ transporter is essential in cells deleted for high-affinity transporter, plasmid pCK64 was used to generate a *trk2Δ* mutation in *trk1Δ* cells. His⁺ transformants of a *trk1Δ his3Δ200* strain (M398) acquired the increased potassium requirement and low-pH hypersensitivity (Fig. 1) described previously for *trk1Δ trk2* cells (21). Southern blot analysis of genomic DNA prepared from the putative *trk1Δ trk2Δ* cells confirmed that these cells carry the large deletion of the *TRK2* gene (Fig. 5). The viability of cells deleted for both the high- and low-affinity K⁺ transporters revealed the existence of additional, functionally independent K⁺ transporter(s). An identical *trk2Δ* allele was constructed in the *trk1Δ/trk1Δ* homozygous diploid Cx142 (Table 1). Following sporulation and tetrad dissection onto YPD(100K) medium, normal viability of the spore colonies was observed. Analysis of nine tetrads showed a 2 K⁺ His⁻:2 K⁻ His⁺ segregation pattern, further confirming that *TRK2* is not essential for viability.

TRK2 mediates low-affinity K⁺ uptake. To measure the relative activities of TRK2 and the transporter(s) remaining

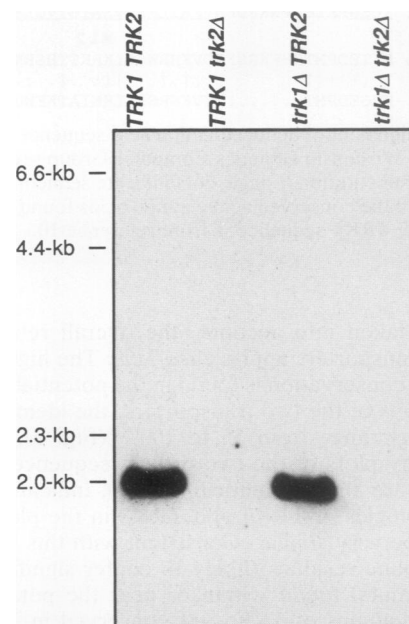


FIG. 5. Southern blot analysis of *TRK1 TRK2*, *TRK1 trk2Δ*, *trk1Δ TRK2*, and *trk1Δ trk2Δ* genomic DNA. Genomic DNA digested with *EcoRV* and *Bam*HI was electrophoresed, transferred to a filter and probed with a ³²P-labeled 2.0-kb *EcoRV* and *Bam*HI fragment from pCK33 (see Materials and Methods for details).

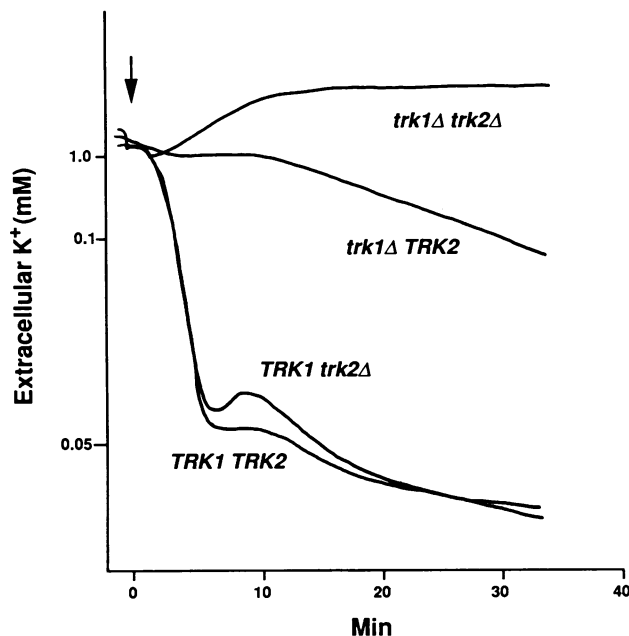
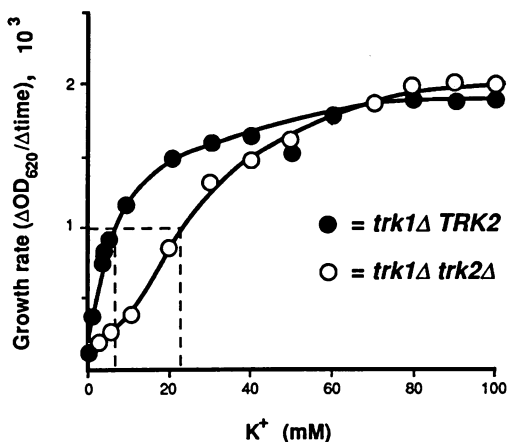


FIG. 6. Potassium uptake assays. Assays were performed by using *TRK1 TRK2* (strain A303), *TRK1 trk2Δ* (strain CY218), *trk1Δ TRK2* (strain M398), and *trk1Δ trk2Δ* (strain M469) cells as described in Materials and Methods. The arrow indicates an addition of glucose to start the assay.

in *trk1Δ trk2Δ* cells, we performed K^+ uptake assays by using isogenic *trk1Δ TRK2* and *trk1Δ trk2Δ* cells. The results of these assays demonstrate that cells containing *TRK2* can take up potassium at a significantly greater rate than cells containing only the remaining transporter(s). When the extracellular potassium in the assay medium was approximately 1 mM, *trk1Δ trk2Δ* cells were unable to take up the ion (Fig. 6). When the extracellular potassium concentration was increased to approximately 15 mM, *trk1Δ trk2Δ* cells showed uptake at a rate approximately one-half that of *trk1Δ TRK2* cells (21, 22).

We quantified the increased K^+ requirement of *trk1Δ trk2Δ* cells compared with *trk1Δ TRK2* cells by measuring the rate of growth in medium containing different potassium concentrations. Compared with *trk1Δ TRK2* cells, *trk1Δ trk2Δ* cells required a 4- to 5-fold increase in extracellular K^+ to achieve half-maximal growth (Fig. 7A). The effect of pH on the growth rate of isogenic *trk1Δ TRK2* and *trk1Δ trk2Δ* cells was also quantified (Fig. 7B). The low-pH-sensitive phenotype was particularly severe at pHs below 4.5. Although the growth rates of *trk1Δ TRK2* and *trk1Δ trk2Δ* cells at pH 5.0 are nearly indistinguishable, at pH 4.0 the generation time of *trk1Δ trk2Δ* cells is nearly double that of *trk1Δ TRK2* cells (230 and 118 min, respectively). Since the low-pH hypersensitivity of *trk1Δ trk2Δ* cells can be suppressed by sufficiently high concentrations of K^+ (21), the K^+ transporter(s) remaining in these cells is unable to take up K^+ efficiently in the presence of a relatively high inward-facing proton gradient. This is in stark contrast to the relative pH insensitivity of K^+ uptake mediated by the *TRK* transporters. The implications of this are discussed below.

A



B

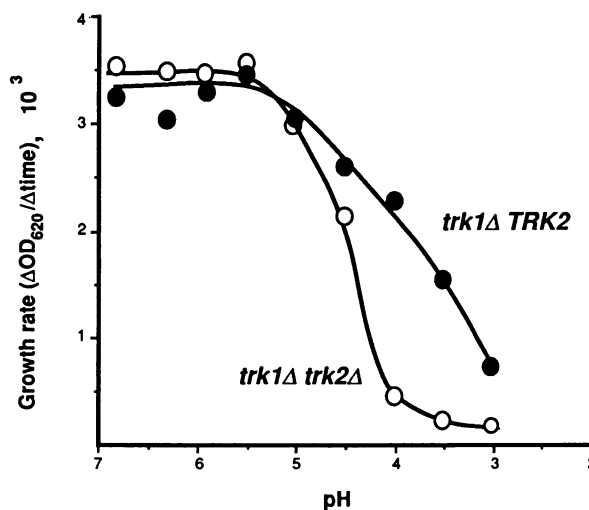


FIG. 7. (A) Determination of the concentration of potassium required to support half-maximal growth ($K_{0.5}$) of *trk1Δ TRK2* and *trk1Δ trk2Δ* cells. The $K_{0.5}$ values for *trk1Δ TRK2* and *trk1Δ trk2Δ* cells are 5 mM and 25 mM KCl, respectively. Isogenic *trk1Δ TRK2* and *trk1Δ trk2Δ* cells (strains M398 and M469) were grown in LS liquid media supplemented with the concentrations of K^+ indicated. A_{620} measurements of cultures were taken hourly until cells entered stationary phase. Each value represents the average of at least three independent samples. (B) Effect of pH on the growth rate of *trk1Δ TRK2* and *trk1Δ trk2Δ* cells. Growth rate was plotted as a function of pH. Isogenic *trk1Δ TRK2* and *trk1Δ trk2Δ* cells (strains M398 and M469) were grown in YPD(100K) at the pHs indicated. A_{620} measurements of cultures were taken hourly until cells entered stationary phase. Each value represents the average of at least three independent samples.

DISCUSSION

In a previous study, we isolated and characterized *TRK1*, the gene likely to encode the high-affinity K⁺ transporter in *S. cerevisiae*. By deleting *TRK1*, a novel cell type was generated (10) that exposed the low-affinity K⁺ transporter to genetic analysis (21). In the present study, we exploited the phenotypes of *trk1Δ trk2* mutants to clone *TRK2*, the gene encoding the putative low-affinity K⁺ transporter. Our results demonstrate that *TRK1* and *TRK2* encode structurally related proteins; the predicted amino acid sequences of *TRK1* and *TRK2* are 55% identical, exhibiting an overall similarity of 71%. Like *TRK1*, *TRK2* contains 12 potential membrane-spanning domains (M1 through M12), with a large hydrophilic region between M3 and M4. The putative membrane-spanning domains (55 to 100% identity) of *TRK1* and *TRK2* share significantly higher sequence conservation than do the remaining regions of the two proteins. Although it is formally possible that *TRK2* encodes a regulatory factor, our data strongly suggest that it encodes a K⁺ transporter.

Although *TRK1* and *TRK2* are highly related, there are significant differences between the two proteins. The 650-amino-acid hydrophilic region between the putative transmembrane domains M3 and M4 in *TRK1* is only 334 amino acids in length in *TRK2*. This, combined with the presence of several other features of *TRK2* that are either deleted or diminished with respect to *TRK1*, suggests that these genes evolved from a duplication event that placed them on separate chromosomes (*TRK1* on chromosome X [10] and *TRK2* on chromosome XI [21]). Once the cell harbored two *TRK* genes, the redundancy and independence of function evidently afforded the opportunity for their evolutionary divergence.

The discovery that *TRK1* and *TRK2* encode structurally homologous K⁺ transporters is another example of ion transporter redundancy that is emerging in yeasts. There are also two genes, *PMA1* and *PMA2*, that encode plasma membrane proton pumps in both *S. cerevisiae* (31, 32, 36) and *Schizosaccharomyces pombe* (12, 13). DNA sequence analysis indicates that the amino acid sequences of the two proton pumps are approximately 70% identical. The genetic redundancy of putative Ca²⁺ transporters in *S. cerevisiae* is carried even further. The *PMR2* locus consists of at least five tandemly repeated, highly related genes likely to encode plasma membrane Ca²⁺-ATPases (28).

TRK2 remains functional despite its divergence, suggesting that the low-affinity K⁺ transporter may play an unknown role in the biology of this organism. Consistent with this hypothesis, we have found that the *TRK2* promoter is unusually large and confers complex regulation on this gene (11). Clues as to why there are multiple transporters of the *TRK* family in *S. cerevisiae* may arise from studies of *TRK2* regulation.

We had previously considered that the high-affinity transporter might function as a K⁺ pump because of the existence of a putative nucleotide-binding domain contained in *TRK1*. The observation that this domain is not present in *TRK2* or in *TRK1* from *Saccharomyces uvarum* (1) suggests that the *TRK* family members are not ATPases. This view is further supported by the observation that overexpression of *TRK2* confers the ability of *trk1Δ* cells to grow on very low levels of potassium (0.2 mM) (11). Since *TRK2* has the ability to fully replace *TRK1* under these circumstances, and since the two proteins are highly related in their primary sequence, it is likely that they function in fundamentally similar ways. For these reasons, we believe that the similarity between the

putative nucleotide-binding domain in *TRK1* and the consensus of authentic nucleotide-binding proteins is fortuitous.

By comparing the inferred amino acid sequences of *TRK1* and *TRK2*, we may determine some of the structural factors that are important for ion transport. These might include the six acidic residues found within the putative transmembrane domains and the highly conserved proline residues in and around these domains. It is likely that the acidic residues form part of the polar pathway that K⁺ ions follow to cross the plasma membrane.

Although the putative transmembrane domains almost certainly harbor the amino acids that directly interact with the potassium ions, it is premature to conclude that the differences in affinity for K⁺ conferred by *TRK1* and *TRK2* are dictated by these regions. It is possible that the transmembrane domains form relatively passive pores through which K⁺ ions are transported and may not be involved in recruitment of potassium ions. We are currently addressing this question through domain swapping experiments to identify specific regions in *TRK1* that confer high-affinity K⁺ uptake when they are constituted in a *TRK1:TRK2* chimeric transporter.

A method for predicting the orientation of the first transmembrane domain in proteins containing multiple membrane-spanning regions has recently been proposed by Hartmann et al. (16). If our model of 12 membrane-spanning domains is correct, when this rule is applied to *TRK2* the N terminus would be cytoplasmic, placing the large hydrophilic domain between M3 and M4 on the outside of the cell. However, if the two conserved sites of potential N-linked glycosylation are in fact glycosylated, this would suggest that the large hydrophilic domain is cytoplasmic.

The viability of *trk1Δ trk2Δ* cells reveals the existence of additional, functionally independent K⁺ transporter(s). This transporter(s) is likely to function very differently from *TRK1* and *TRK2* since, by comparison, *trk1Δ trk2Δ* cells are hypersensitive to the presence of protons in the growth medium. Growth of *trk1Δ trk2Δ* cells is severely inhibited in medium adjusted below pH 4.0, even in the presence of potassium concentrations that approximate the internal concentration of this ion (3, 24). Since high concentrations of potassium or rubidium but not sodium or sorbitol can suppress the low-pH hypersensitivity (21), it appears that the transporter(s) in *trk1Δ trk2Δ* cells is unable to take up K⁺ in a low-pH medium. When faced with large extracellular proton gradients, this transporter(s) is effective only when K⁺ uptake is not driven against its chemical gradient. This limitation distinguishes this transporter(s) from the *TRK* family of transporters, since the latter is essentially resistant to the effects of low extracellular pH on K⁺ uptake and can transport K⁺ against a 1,000-fold concentration gradient.

By deleting *TRK1* and *TRK2*, we have generated cells that depend on the function of K⁺-transporting proteins that normally do not function to supply the cell with the bulk of its required K⁺ ions. Rather than performing an ion recruitment function, the transporter(s) in *trk1Δ trk2Δ* cells may mediate K⁺ transport primarily to regulate pH homeostasis or the electrical potential or, conceivably, to couple the uptake of another molecule to that of potassium. Several mechanisms are consistent with the reduced rate of K⁺ uptake and the hypersensitivity to low pH that appear to be exhibited by *trk1Δ trk2Δ* cells. First, a K⁺/H⁺ antiporter similar to the transport mechanism reported to occur in *Neurospora crassa* (2) could couple the uptake of K⁺ to the efflux of protons and, as a consequence, would be significantly affected by the extracellular proton concentration.

Second, K⁺ channels have been functionally identified in *S. cerevisiae* by electrophysiological techniques (14, 15), but as yet they remain genetically unidentified. Conceivably, high proton concentrations could either reduce the likelihood of the open channel state or inhibit K⁺ uptake by competitive inhibition. Finally, transporters of nutrients such as sugars and amino acids are thought to function via the cotransport of protons. It is possible that such transporters accommodate K⁺ with much lower affinity than protons. In all of these scenarios, the cell would still rely on the plasma membrane ATPase to generate the electrical potential required to drive K⁺ uptake. We have isolated dominant pseudorevertants that reduce the K⁺ requirement of *trk1Δ trk2Δ* cells (22). The analysis of these mutants is now underway in an effort to determine which, if any, of these possibilities is correct.

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